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Prostaglandin E_2 as a Modulator of Macrophage-T Lymphocyte Interactions

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Sufficient data exist to implicate the monocyte-macrophage as the major source of prostaglandins in monocyte-macrophage-T lymphocyte interactions. Prostaglandin (PG) E_2, a major prostaglandin synthesized by human monocyte-macrophages is able to modulate a variety of T lymphocyte reactivities including blastogenesis, lymphokine secretion, and cytotoxicity. The effect of PGE_2 on T lymphocyte function varies according to the primary activating stimulus, the specific response under investigation, as well as the population of T lymphocytes involved. Some immunologic abnormalities found in clinical disease may in part derive from alterations occurring in the synthesis of PGE_2 by monocyte-macrophages or in the response of T lymphocytes to PGE_2.

Cells belonging to the monocyte-macrophage line (subsequently designated as MΦs) perform a variety of immunological functions required for the normal reactivity of T lymphocytes. These include the appropriate presentation of antigenic determinants as well as the synthesis and release of various soluble factors which serve to enhance or suppress T lymphocyte reactivity (reviewed in reference 1). Included among these soluble factors are certain members of the prostaglandin (PG) family which consists of a group of oxygenated cyclopentane derivatives synthesized from polyunsaturated fatty acids (reviewed in references 2 and 3). This paper will discuss the concept of MΦ-dependent modulation of T lymphocyte reactivity through the synthesis of PGE_2.

NATURE OF THE PGE_2-PRODUCING CELL

The frequently repeated statement that PGs are ubiquitous products of cellular metabolism has lead to the assumption that all cells synthesize these products. However, existing studies suggest that, in the context of MΦ-T lymphocyte interactions, the synthesis of PGE_2 is a property of the MΦ but not of the T lymphocyte. For example, deletion of MΦs from human peripheral blood mononuclear cells or from animal spleen cells leads to a loss in the recovery of PGE_2 from the non-MΦ mononuclear cells whereas the isolated MΦs readily synthesize PGE_2 (4–10). Moreover, removal of T lymphocytes from murine spleen cell preparations fails to alter the levels of PGE_2 generated by the whole spleen cell preparation [6]. The Figure shows thin-layer radiochromatographic data from our laboratory demonstrating that human peripheral blood MΦs (>95% esterase positive) but not T lymphocytes (>90% T cells) are able to convert the radiolabeled PG precursor ^{14}C-arachidonic acid into ^{14}C-PGE_2.

We have recently found that PGE_2 synthesis among human peripheral blood MΦs reflects a heterogeneous contribution from different MΦ subpopulations, some of which release substantially more than do others [11]. While the significance of the heterogeneity is currently unclear, the data imply that functionally, not all MΦs have equivalent potential for providing PGE_2 as a regulatory product. Previous studies have demonstrated that populations of human MΦs also differ with regard to other immunoregulatory capabilities [12].

Studies from several laboratories, including our own, have shown that in addition to PGE_2, human MΦs also synthesize PGE_1 and $PGF_{2\alpha}$ as well as the PG-related thromboxane B_2 (although the presence of thromboxane as a platelet-derived contaminant has not been completely excluded) [7,10,11]. There is conflicting data with regard to the synthesis of other PGs (ie. PGD_2, PGI_2 (prostacyclin) etc.) by MΦs probably due to variations in sensitivity of the assays employed or to the purity and source of the MΦs assayed [10,13]. However, PGE_2 has so far been the major PG associated with having an immunoregulatory potential (reviewed in reference 3). PGE_1 has, in general, been found to be equally as potent as PGE_2, but the levels generated by human peripheral blood MΦs are more than 10-fold lower than those of PGE_2 [11]. This value is consistant with the finding that arachidonic acid, the precursor of the 2-series PGs (ie., PGE_2, $PGF_{2\alpha}$ etc.) constitutes approximately 20% of the fatty acids esterified to human MΦ membrane phospholipids whereas dihomo-γ-linolenic acid, the precursor of the 1-series PGs (ie. PGE_1, $PGF_{1\alpha}$, etc) appears to constitute less than 1% of the esterified fatty acids [15]. $PGF_{2\alpha}$, at least in the experimental models studied, has not exhibited immunoregulatory activity.

The Table lists the various agents capable of stimulating PGE_2 synthesis by human peripheral blood MΦs. Studies involving animal MΦs have shown, in addition, that purified protein derivative and partially purified macrophage inhibitory factor (MIF), a lymphokine, are also stimulatory [16]. Despite the variety of factors capable of stimulating *in vitro* PG synthesis by MΦs, some specificity in the nature of the stimuli does appear to exist since a lymphocyte mitogen such as concanavalin A (Con A) directly induces PGE_2 synthesis by human peripheral blood MΦs whereas the mitogens phytohemagglutinin (PHA) and pokeweed mitogen (PWM) apparently do not [9]. However, PHA and PWM have been reported to stimulate PGE_2 synthesis by whole peripheral blood mononuclear cells [4,18]. These observations could be explained by the fact that PHA is capable of releasing free arachidonic acid from peripheral blood lymphocytes [19] which although incapable of synthesizing PGs themselves, would provide the necessary substrate for PGE_2 synthesis by the resident MΦs. This possibility is strongly supported by the observation that ^{14}C-arachidonic acid is converted to PGE_2 by isolated peripheral blood MΦs but not by peripheral blood T lymphocytes or by the other remaining peripheral blood mononuclear cells (see the Figure). Thus the T lymphocyte could potentially regulate its own functions by stimulating PGE_2 synthesis in MΦs either through the release of lymphokines such as MIF [16] or by supplying the free substrate to the MΦs for PGE_2 synthesis.

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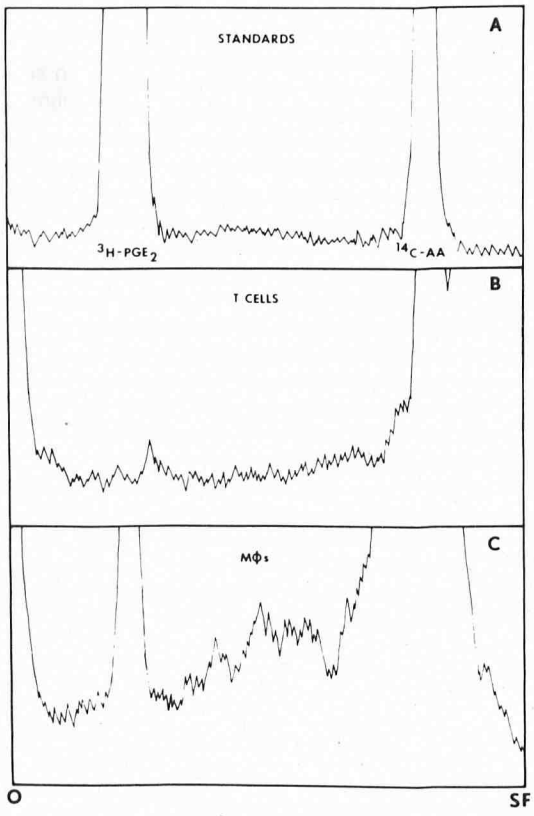
Abbreviations:
MIF: macrophage inhibitory factor
PG: prostaglandin
PHA: phytohemagglutinin
PWM: pokeweed mitogen

Factors affecting PGE_2 synthesis by human peripheral blood MΦs

Stimulation	No effect
Aggregated myeloma IgG [9]	Albumin [9]
Antigen-antibody complexes [17]	IgG [9]
Concanavalin A [9]	Fab fragments [9]
Cytochalasin B [9]	Latex particles [9]
Endotoxin [8,9]	Phytohemagglutinin [9]
Fc fragments [9]	Pokeweed mitogen [9]

Numbers refer to references in bibliography.



PGE_2 synthesis by human T cells and MΦs. Human peripheral blood T cells and MΦs were isolated as previously described [12,28]. Cells were incubated in tris buffer pH 7.4 containing 0.05mM EDTA for 30 min at 37°C following the addition of 1.3 μg (500,000 dpm) of [1-^{14}C] arachdonic acid (AA). Ether extracts of acidified culture media (pH 3) were subjected to thin-layer chromatography on silica gel G using the organic phase of the solvent system: ethyl acetate:2,2,4-trimethylpentane:acetic acid:water (44:20:8:40). Radioactive peaks were detected using a Berthold LB2760 TLC scanner. A: Standards of [5, 6, 8, 11, 12, 14, 15(n)-^{3}H] PGE_2, sp.act. 160Ci/mmol; [1-^{14}C] AA, sp. act. 56.5mCi/mmol. B. 20×10^6 T cells. C. 20×10^6 Mϕs. O = origin; SF = solvent front; vertical axis = relative radioactivity.

MODULATION OF T LYMPHOCYTE REACTIVITY BY PGE_2

The *in vitro* effects of PGE_2 on such T lymphocyte responses as mitogen-induced blastogenesis [5,18–22], lymphokine secretion [16,23–25] and cytotoxicity [26,27] have been well documented. While the most frequently observed effect of PGE_2 on these responses has been inhibitory, the effect on the release of a lymphokine such as osteoclast activating factor has been stimulatory [24]. Furthermore, recent studies from our laboratory have shown that, even in regard to a single T lymphocyte response such as mitogen-induced blastogenesis, the modulatory effect of PGE_2 depends on the T lymphocyte subpopulation in question [28]. We observed that subpopulations of human peripheral blood T lymphocytes derived from peripheral blood mononuclear cells by density gradient fractionation show diametrically opposite responses to PGE_2 following mitogenic stimulation with PHA; that is, the blastogenic response of T lymphocytes segregating to a low-density region of the gradient is enhanced by PGE_2 whereas the response of cells segregating to a higher density region of the gradient is suppressed. The degree of blastogenic suppression caused by PGE_2 in the unfractionated T lymphocytes was roughly the algebraic sum of the individual reactivities of the subpopulations.

While the studies cited above involve the addition of exogenous PGE_2 to the responding cells, several observations suggest that endogenous production of PGE_2 by MΦs is involved in the normal regulation of the primary responses studied. First, removing the MΦs from the experimental system or adding inhibitors of PG synthesis without removing the MΦs alter the normally observed (control) T lymphocyte response in the direction expected by the removal of PGE_2 [5,22]. Second, the levels of PGE_2 recovered from the various *in vitro* systems studied (10^{-8}–10^{-7} M) fall within the range which when added exogenously modulate T lymphocyte reactivity [10,11,16,18, 28].

It is important to remember that the effects of PGE_2 on T lymphocyte reactivity are modulatory in nature, altering a response already initiated by some other primary stimulus. Furthermore, the effect of PGE_2 on T lymphocyte reactivity can vary and must, therefore, be defined in the context of the primary stimulus [21,22], the specific reactivity under study [23,24] and the population or subpopulation of T lymphocyte in question [28].

CLINICAL CORRELATIONS

Based on the data reviewed above, it could be postulated that changes in PGE_2 production by MΦs or in T lymphocyte response to MΦ-derived PGE_2 could result in altered immunologic reactivity of T lymphocytes in clinical disease. In fact, evidence has been presented that the hyporesponsiveness of T lymphocytes to PHA in anergic patients with Hodgkin's disease results in part from the excessive synthesis of PGE_2 by the peripheral blood MΦs [29]. Blocking PG synthesis with indomethacin or removing the PGE_2 - producing MΦs from the PBMC restored the blastogenic response virtually to control values. A fourfold increase in PGE_2 by the whole peripheral blood mononuclear cells was documented. Other investigators have described a similar phenomenon but have shown that the increased levels of PGE_2 produced by mononuclear cells from patients Hodgkin's disease result from an increase in the absolute number of peripheral blood MΦs [30]. Additional data suggest that the peripheral blood lymphocytes in Hodgkin's disease may also induce greater synthesis of PGE_2 from MΦs [30]. This could conceivably result from an increased release of free arachidonic acid or PG-stimulating lymphokine (ie., MIF—reference 16) by the lymphocytes of these patients. While other studies demonstrate that non-PG related suppression of T lymphocyte blastogenesis may occur in Hodgkin's disease [31,32], increased PGE_2 synthesis by MΦs may indeed represent one factor contributing to altered T lymphocyte reactivity in these patients. A similar situation has been described in patients with active sarcoidosis demonstrating depressed cell-mediated immunity [33].

Whether or not the restoration of the *in vitro* blastogenic response of T lymphocytes by the use of PG synthesis inhibitors leads to a reversal of clinical anergy has not yet been documented in the patients with Hodgkin's disease or active sarcoidosis. However, a report has been made documenting such a reversal of both clinical anergy and suppressed *in vitro* blastogenesis in a patient with adult acquired hypogammaglobulinemia [34]. Interestingly, reversal was noticed only during the period of indomethacin therapy.

Studies on other diseases involving altered immunologic status also show changes in the response of T lymphocytes to

PGE_2. For example, in multiple sclerosis, while T lymphocyte blastogenesis is sensitive to suppression by PGE_2 [35], the release of the lymphokine LIF (leukocyte migration inhibitory factor), unlike in cells from normal individuals, appears resistant to PGE_2-mediated suppression [36]. It has been hypothesized that a similar refractoriness of T lymphocytes to the modulatory effects of PGE_2 could explain certain altered immunologic responsiveness observed in patients with rheumatoid arthritis [37].

Clearly, further investigation is required to define the roles of PGE_2 and possibly other MΦ-derived PGs or related products (thromboxanes, hydroxy fatty acids, and possibly leukotrienes [38]) in both physiologic and pathophysiologic aspects of MΦ-T lymphocyte interactions. Nevertheless, the data presented and reviewed in this paper strongly implicate PGE_2 as an important agent through the release of which MΦs may modulate T lymphocyte reactivity.

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DISCUSSION

DAVIES: How do you characterize your peripheral blood mononuclear phagocyte subpopulations?

GOLDYNE: In our studies, the subpopulations of monocytes are only defined by where they sediment in a discontinuous BSA density gradient. The identification of these subpopulations as monocytes is made on the basis of esterase positivity, adherence, and phagocytosis.

DAVIES: What is the minimum period of time needed for responding lymphocytes to be exposed to exogenous PGE_2 in order to demonstrate an effect on ^{3}H-Tdr incorporation?

GOLDYNE: I do not believe that that parameter has been examined. Webb and Nowowiejski (*Cell Immunol* 41:72–85, 1978) however, showed that PGE_2 suppression of lymphocyte blastogenesis occurred only if the PGE_2 was added between 0 and 24 hr after mitogen stimulation. More recently, Muscoplat and colleagues (*Infect Immunol* 26:311–315, 1979) showed that maximum suppression of lymphocyte blastogenesis by PGE_2 occurred only if the prostaglandin was added within 4 hr following exposure to PHA but that by 24 hr after PHA exposure PGE_2 could no longer affect the blastogenic reactivity. However, this response pattern could be unique to PHA and ConA since these authors demonstrated that using PPD as the mitogen, PGE_2 was still able to suppress mitogenesis significantly even when added at 24 hr after PPD exposure. Furthermore, in light of our studies, it would appear that the loss of PGE_2-induced blastogenic suppression in response to PHA in fact represents the masking of a continuous suppressability of one subpopulation of T cells by an increased blastogenic response induced by PGE_2 in another subpopulation of T cells.

DAVIES: But have any experiments been done where the lymphocytes have been pulsed with exogenous prostaglandin and then the

prostaglandin taken away and then the cells examined to see whether there is a long lasting effect of that pulse.

GOLDYNE: I am not aware of a study that has carried out that particular experiment with blastogenic reactivity.

LICHTENSTEIN: Several years ago, Henry Bourne, Chris Henney and I showed that the addition of PGE_2 elevated cAMP quite rapidly and the level also fell rapidly when PGE_2 was washed away. PGE_2 inhibits T cell cytotoxicity and this inhibitory effect follows the cAMP level, ie., if you wash PGE away, the inhibition is also lost.

GOLDYNE: What I see as important in the context of an evolving understanding of prostaglandin function is that the old dogma that PGE_2, for example, has only one effect on a given cell system (ie. T lymphocytes) has evolved because the heterogeneity of such cell systems in terms of response to prostaglandins has only recently begun to be considered and appropriately examined. Our awareness of such heterogeneity in response to a given prostaglandin may lead to a better understanding of the roles of prostaglandins in cellular interactions.

GREEN: Ordinary immunologists should be more concerned with prostaglandin production by macrophages. The production of prostaglandins may influence many types of immunological reactions, *in vitro*, in which macrophages are present and confuse the interpretation of the results of proliferative as well as cytotoxic assays.

COHN: What is the half life of PGE_2 in these cultures? Is most of it bound to albumin?

GOLDYNE: When we added tritiated PGE_2 to the monocyte cultures, no noticeable breakdown could be appreciated by thin-layer chromatography at 24 hr after addition so that the half life in this *in vitro* system appears to be fairly long. In regard to PGE_2 binding to albumin, Gueriguian (*J Pharmacol Exp Therap* 197:391–401, 1976), Raz (*Biochim Biophys Acta* 280:602–613, 1972) and Unger (*J Pharm Pharmacol* 24:470–477, 1972) have demonstrated a weak, noncovalent binding between PGE_2 and serum albumin. From Unger's data on equilibrium dialysis of ^{3}H-Tdr PGE_1 against various concentrations of albumin, very little, ie. less than 5%, would be bound to albumin in our cultures where we have only 5–10% fetal calf serum.

CLARK: Upon routine isolation of mononuclear cells, I have been intrigued by the observation that platelets often rosette around mononuclear cells (?monocytes). Would you comment further on your findings of thromboxane production by macrophages?

GOLDYNE: All that I can say is that we too have noticed this phenomenon of platelet adherence to the monocytes, and the thromboxane synthesis by these monocytes could possibly account for this observation.

AUSTIN: What are the functional and/or biological correlations of high and low density T cells? Do the PGE_2 responsive cells have lower levels of baseline cAMP?

GOLDYNE: We are just beginning to examine the possible functional correlations of the high and low density T cell populations. The low density T cells whose blastogenic reactivity was enhanced by PGE_2 had a baseline level of cAMP roughly twice that of the high density cells whose blastogenic reactivity was suppressed by PGE_2.

LICHTENSTEIN: In fact you showed that high density lymphocytes were inhibited by PGE_2 and low density lymphocytes were enhanced. Both cell preparations showed about 2.5 fold increase in cAMP after PGE_2. The critical point, I think, was that the low density cells started with approximately twice the cAMP level of the high density cells so that while the percentage increase was similar, the absolute levels were quite different.